I claim:

1. A process for producing an antibiotic which comprises placing cells of Lactobacilius reuteri capable of producing the antibiotic under conditions favorable for production of the antibiotic.

2. A process for producing an antibiotic according to claim 1 in which the favorable conditions comprise the presence of glycerol and a reduced oxygen tension.

3. A process for producing an antibiotic according to claim 2 which the glycerol concentration is 20-500 mM and the L. reuteri cells are incubated at 37 degrees C in still culture.

4. A process for producing an antibiotic according to claim 1 in which the favorable conditions comprise the presence of glyceraldehyde and a reduced oxygen tension.

5. A process for producing an antibiotic according to claim 2 in which the favorable conditions comprise the presence of heterologous microorganisms in the still culture.

6. A process for producing an antibiotic which comprises placing cells of a strain of Lactobacillus reuteri capable of producing the antibiotic in glycerol solution under conditions of reduced oxygen until substantial activity is imparted to the solution and isolating the antibiotic so produced in substantially pure form.

7. The process for producing an antibiotic according to claim 6 wherein the isolation comprises the steps of:

(a) separating the L. reuteri cells from a sample of the solution having antibiotic activity;

(b) analyzing the sample using high performance liquid chromatography;

(c) eluting of the sample; and

(d) collecting the material eluting from a peak intermediate between the peaks for the reference standards for 1,3-propanediol and glycerol.

8. A method for determining the presence of an antibiotic in a solution of Lactobacillus reuteri comprising the steps of:

(a) separating the L. reuteri cells from a sample of the solution;

(b) analyzing the sample using high performance liquid chromatography;

(c) eluting of the sample; and

(d) determining the presence of a peak intermediate in elution time between those of the reference standards for 1,3-propanediol and glycerol by monitoring for refractive index changes.

9. A method for screening Lactobacillus reuteri isolates to identify those that produce an antibiotic which comprises the steps of:

(a) inoculating a suspension of microorganisms from an animal source on a solid Lactobacillus growth medium;

(b) incubating said inoculated growth medium under conditions that promote growth of Lactobacillus colonies;

(c) replicating the Lactobacillus colonies;

(d) overlaying the inoculated growth medium with a liquified semisolid mixture containing a suspension of a living test microorganism and a carbon source selected from the group consisting of glycerol and glyceraldehyde;

(e) incubating the overlaid inoculated medium under conditions that promote growth of the test microorganism; and

(f) identifying in situ those Lactobacillus colonies that produce the antibiotic by detecting zones of growth inhibition surrounding said colonies.

10. The method for screening Lactobacillus reuteri isolates of claim 9 wherein the Lactobacillus growth medium is made to be

highly selective for lactobacilli by addition of sodium acetate and adjustment of the medium pH to 5.5, the carbon source is glycerol and the living test microorganism is a Lactobacillus plantarum.

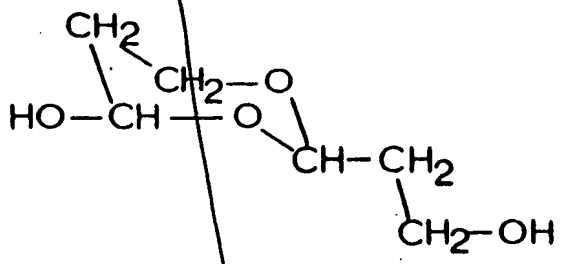
11. The method for screening Lactobacillus reuteri isolates of claim 10 wherein the conditions of incubation of the inoculated growth medium comprise an incubation temperature of 37 degrees C for 48 hours at a reduced oxygen tension.

12. An antibiotic and derivatives thereof, effective in inhibiting Gram-positive and Gram-negative bacteria and the eucaryotic organisms, Saccharomyces cerevisiae and Trypanosoma cruzi, containing the elements carbon, hydrogen and oxygen in substantially the following percentages by weight: carbon, 48.65%, hydrogen, 8.11%; oxygen, 43.24%; having a molecular weight of approximately 148 grams per mole; being soluble in water, nonresistant to heat at pH 9.0 and resistant to proteases and nucleases; and exhibiting characteristic elution with water or 0.01 M H_2SO_4 between 1,3-propanediol and glycerol on high performance liquid chromatography.

13. An antibiotic as defined in claim 12 in its substantially pure form.

14. An antibiotic as defined in claim 13 having the formula $C_6H_{12}O_4$.

15. An antibiotic of the formula:



16. A method for inhibiting microorganism growth comprising exposing the microorganisms to an antibiotic produced by Lactobacillus reuteri cells.

17. A biologically pure strain of Lactobacillus reuteri ATCC No. 53608 (Strain 1063).

18. A method of inhibiting viral production comprising exposing viruses to an antibiotic produced by Lactobacillus reuteri cells.

19. A method for increasing the number of Lactobacillus reuteri cells in the gastro-intestinal tract of animals and optimizing the conditions for antibiotic production by the Lactobacillus reuteri cells, which method comprises feeding the animals Lactobacillus reuteri cell cultures.

20. A method for increasing the number of Lactobacillus reuteri cells in the gastro-intestinal tract of animals and optimizing the conditions for antibiotic production by the Lactobacillus reuteri cells according to claim 19, further comprising feeding the animals substances that are conducive to Lactobacillus reuteri antibiotic production.

21. A method of inhibiting ribonucleotide reductase activity and the DNA synthesis dependent on the ribonucleotide reductase activity comprising exposing the ribonucleotide reductase to an antibiotic produced by Lactobacillus reuteri cells.

22. A method for inhibiting microorganism growth comprising exposing the microorganisms to an antibiotic according to claim 12.

23. A method for inhibiting microorganism growth comprising exposing the microorganisms to an antibiotic according to claim 14.

24. A method of inhibiting viral production comprising exposing viruses to an antibiotic according to claim 12.

25. A method of inhibiting viral production comprising exposing viruses to an antibiotic according to claim 14.

26. A method for inhibiting microorganism growth according to claim 22, wherein the microorganism growth is inhibited in foods.

27. An antimicrobial food preservative comprising the antibiotic of claim 12.

28. An antimicrobial food preservative comprising the antibiotic of claim 14.

29. A method for inhibiting microorganism growth according to claim 22, wherein the microorganism growth is inhibited in animals.

30. A method for biologically synthesizing 4-hydroxy-2-2'-hydroxyethyl-1:3-dioxan comprising placing Lactobacillus reuteri cells under conditions favorable for the production of 4-hydroxy-2-2'-hydroxyethyl-1:3-dioxan.

31. A method for biologically synthesizing β-hydroxypropaldehyde comprising placing Lactobacillus reuteri cells under conditions favorable for the production of β-hydroxypropaldehyde.

AMENDED CLAIMS

[received by the International Bureau on 12 September 1988 (12.09.88) original claims 22 - 26 , 28 and 29 replaced by amended claims 22 - 26 , 28 and 29 ; claim 27 cancelled ; other claims unchanged (4 pages)]

~~16. A method for inhibiting microorganism growth comprising~~ exposing the microorganisms to an antibiotic produced by Lactobacillus reuteri cells.

17. A biologically pure strain of Lactobacillus reuteri ATCC No. 53608 (Strain 1063).

18. A method of inhibiting viral production comprising exposing viruses to an antibiotic produced by Lactobacillus reuteri cells.

19. A method for increasing the number of Lactobacillus reuteri cells in the gastro-intestinal tract of animals and optimizing the conditions for antibiotic production by the Lactobacillus reuteri cells, which method comprises feeding the animals Lactobacillus reuteri cell cultures.

20. A method for increasing the number of Lactobacillus reuteri cells in the gastro-intestinal tract of animals and optimizing the conditions for antibiotic production by the Lactobacillus reuteri cells according to claim 19, further comprising feeding the animals substances that are conducive to Lactobacillus reuteri antibiotic production.

21. A method of inhibiting ribonucleotide reductase activity and the DNA synthesis dependent on the ribonucleotide reductase activity comprising exposing the ribonucleotide reductase to an antibiotic produced by Lactobacillus reuteri cells.

32

~~22~~. A method for inhibiting microorganism growth comprising exposing the microorganisms to an antibiotic, said antibiotic comprising an antibiotic and derivatives thereof, effective in inhibiting Gram-positive and Gram-negative bacteria and the eucaryotic organisms, Saccharomyces cerevisiae and Trypanosoma cruzi, containing the elements carbon, hydrogen and oxygen in substantially the following percentages by

Rule 126

weight: carbon, 48.65%; hydrogen, 8.11%; oxygen, 43.24%; having a molecular weight of approximately 148 grams per mole; being soluble in water, nonresistant to heat at pH 9.0 and resistant to proteases and nucleases; and exhibiting characteristic elution with water or 0.01 M H_2SO_4 between 1,3-propanediol and glycerol on high performance liquid chromatography.

33. A method for inhibiting microorganism growth comprising exposing the microorganisms to an antibiotic according to claim 32, said antibiotic having the formula $C_6H_{12}O_4$, said antibiotic being in its substantially pure form.

34
24. A method of inhibiting viral production comprising exposing viruses to an antibiotic, said antibiotic comprising an antibiotic and derivatives thereof, effective in inhibiting Gram-positive and Gram-negative bacteria and the eucaryotic organisms, Saccharomyces cerevisiae and Trypanosoma cruzi, containing the elements carbon, hydrogen and oxygen in substantially the following percentages by weight: carbon, 48.65%; hydrogen, 8.11%; oxygen, 43.24%; having a molecular weight of approximately 148 grams per mole; being soluble in water, nonresistant to heat at pH 9.0 and resistant to proteases and nucleases; and exhibiting characteristic elution with water or 0.01 M H_2SO_4 between 1,3-propanediol and glycerol on high performance liquid chromatography.

35
25. A method of inhibiting viral production comprising exposing viruses to an antibiotic according to claim 34, said antibiotic having the formula $C_6H_{12}O_4$, said antibiotic being in its substantially pure form.

Rule 126

36
26. A method for inhibiting microorganism growth in foods comprising exposing the microorganisms to an antibiotic, said antibiotic comprising an antibiotic and derivatives thereof, effective in inhibiting Gram-positive and Gram-negative bacteria and the eucaryotic organisms, Saccharomyces cerevisiae and Trypanosoma cruzi, containing the elements carbon, hydrogen and oxygen in substantially the following percentages by weight: carbon, 48.65%; hydrogen, 8.11%; oxygen, 43.24%; having a molecular weight of approximately 148 grams per mole; being soluble in water, nonresistant to heat at pH 9.0 and resistant to proteases and nucleases; and exhibiting characteristic elution with water or 0.01 M H_2SO_4 between 1,3-propanediol and glycerol on high performance liquid chromatography.

37
28. A method for inhibiting microorganism growth in foods comprising exposing the microorganisms to an antibiotic according to claim 26, said antibiotic having the formula

$C_6H_{12}O_4$, said antibiotic being in its substantially pure form.

38
~~29~~. A method for inhibiting microorganism growth, wherein the microorganism growth is inhibited in animals by exposing the microorganisms to an antibiotic comprising an antibiotic and derivatives thereof, effective in inhibiting Gram-positive and Gram-negative bacteria and the eucaryotic organisms, Saccharomyces cerevisiae and Trypanosoma cruzi, containing the elements carbon, hydrogen and oxygen in substantially the following percentages by weight: carbon, 48.65%; hydrogen, 8.11%; oxygen, 43.24%; having a molecular weight of approximately 148 grams per mole; being soluble in water, nonresistant to heat at pH 9.0 and resistant to proteases and nucleases; and exhibiting characteristic elution with water or 0.01 M H_2SO_4, between 1,3-propanediol and glycerol on high performance liquid chromatography.

39
~~30~~. A method for biologically synthesizing 4-hydroxy-2'-hydroxyethyl-1:3-dioxan comprising placing Lactobacillus reuteri cells under conditions favorable for the production of 4-hydroxy-2-2'-hydroxyethyl-1:3-dioxan.

40
~~31~~. A method for biologically synthesizing β-hydroxypropaldehyde comprising placing Lactobacillus reuteri cells under conditions favorable for the production of β-hydroxypropaldehyde.

add B7

add F7

add E7

STATEMENT UNDER ARTICLE 19

Please cancel original pages 43-44 of this application, and substitute therefor the enclosed new pages 43, 43a, 44 and 44a. Claims 1-15 on pages 40-42 of the application as filed have not been amended.

Claims 16-21 on the enclosed pages are identical to the same numbered claims previously on file.

New Claim 22 corresponds to prior Claim 22, to which has been added the recitation of Claim 12 rather than the reference to Claim 12.

New Claim 23 corresponds to prior Claim 23, to which has been added the recitation of Claim 14 rather than the reference to Claim 14 and the recitation of Claim 13 that is referred to in Claim 14.

New Claim 24 corresponds to prior Claim 24, to which has been added the recitation of Claim 12 rather than the reference to Claim 12.

New Claim 25 corresponds to prior Claim 25, to which has been added the recitation of Claim 14 rather than the reference to Claim 14 and the recitation of Claim 13 that is referred to in Claim 14.

New Claim 26 corresponds to prior Claim 26, to which has been added the recitation of Claim 12 rather than reference to Claim 22 which in turn refers to Claim 12.

Claim 27 has been cancelled.

New Claim 28 corresponds to prior Claim 28, to which has been added the recitation of Claim 14 rather than reference to Claim 14 and the recitation of Claim 13 that is referred to in Claim 14.

New Claim 29 corresponds to prior Claim 29, to which has been added the recitation of Claim 22 which refers to Claim 12, rather than reference to Claim 12.

Claims 30 and 31 are identical to the same numbered claims previously on file.

The claims as amended are fully consistent with and supported by the as-filed disclosure of the application as each incorporates by complete recitation that which was previously referred to in the prior claims.